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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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Additional inventors are being named on the Additional Page *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

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Respectfully submitted,

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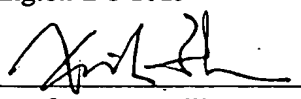
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UNITED STATES PROVISIONAL PATENT APPLICATION

For

FLUIDIC DEVICES AND METHODS FOR MULTIPLEX CHEMICAL AND BIOCHEMICAL REACTIONS

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UNITED STATES PROVISIONAL PATENT APPLICATION

[0001] TITLE: Fluidic Devices and Methods for Multiplex Chemical and Biochemical Reactions

[0002] INVENTORS: Xiaochuan Zhou, Xiaolian Gao, Erdogan Gulari

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0003] Not applicable

REFERENCE TO A "Microfiche Appendix"

[0004] Not applicable.

BACKGROUND OF THE INVENTION

1. FIELD OF THE INVENTION

[0005] The present invention relates to the field of fluidic devices for carrying out multiplex chemical or biochemical reactions and for performing multiplex chemical and/or biochemical assays. More particularly, this invention relates to devices and methods for distributing fluids, segregating fluids, carrying out chemical and/or biochemical reactions, and detecting chemical or biochemical products.

2. DESCRIPTION OF RELATED ART

[0006] Modern drug development, disease diagnosis, pathogen detection, gene discovery, and various genetic-related technologies and research increasingly rely on making, screening, and assaying a large number of chemical and/or biochemical compounds. Traditional methods of making and examining the compounds one at a time are becoming increasingly inadequate. Therefore there is a need for chemical/biochemical reaction systems and devices to perform high-throughput assay and synthesis.

[0007] One of the most commonly used high-throughput multiplexing method relies on the use of titer plates. Each titer plate contains 96, 384, or 1,536 microwells or microtubes in which individual chemical and/or biochemical reactions are carried out. (need a reference) In a standard format the reaction media inside individual microwells or microtubes are physically

isolated from each other. Chemical and biochemical reagents are delivered into the microwells or microtubes either robotically or manually using pipettes or dispensers. In a standard format the distances between adjacent microwells or microtubes are 9.0 mm, 4.5 mm, and 2.25 mm for 96, 384, and 1,536 microwell titer plates, respectively. To increase throughput, higher densities of the microwells are needed.

[0008] Another multiplexing method relates to microarrays. The most well-known microarray is DNA microarray, which, in its most common form, is a glass plate containing a two-dimensional array of DNA materials on its surface. A DNA microarray is used as a multiplexing detection device. Each element of the array has a unique DNA sequence, which is used to specifically recognize or detect a unique complementary DNA sequence in a sample solution. The element density of a DNA microarray is usually much higher than that of a titer plate. On a commercially available DNA microarray the distance between two adjacent elements is between 10 micrometer and 500 micrometer. DNA microarray, are rapidly becoming fundamental tools in genomic, proteomic, and other biological research.[1,2,3] In addition to research use, DNA microarray has the potential to be used as a clinical diagnostic tool.[4,5] In addition to DNA microarray there are various other types of microarrays, such as peptide microarray (Lian's Nature Biotech paper), protein microarray (need a reference), and tissue microarray (need a reference), for various research and diagnostic applications.

[0009] Microarray technology has fundamentally changed the way of studying biological systems from observing one or a few genes or molecular species at a time to observing pathways, networks, and molecular machines that involve the interplay of a large collection of genes and pools of molecules. DNA microarray chips available today operate based on the hybridization of target DNA or RNA molecules in a solution phase (the sample to be tested) with probe DNA (oligonucleotides or cDNA) molecules immobilized on solid substrates (in either plate or bead forms).[6] The hybridization results are used in monitoring gene expression, determining nucleotide sequences, identifying gene mutations, detecting pathogens, and selecting and measuring activities of ligand molecules (peptides, proteins, antibiotics and other organic and inorganic molecules).[1,2,3]

[0010] In spite of the usefulness of the currently available DNA microarrays, their performance is far from being satisfactory for many applications. Inadequate assay specificity is

one of a multitude of limitations with the current DNA microarray methodology, which are fundamentally associated with the single-pair hybridization assay, i.e. with results determined by the hybridization of only one pair of nucleotide molecules. Assay specificity relies on hybridization discrimination, which in turn is determined by probe (immobilized DNA) sequence design, probe sequence purity, target (sample DNA) sequence composition, and hybridization conditions. Selection of hybridization probes is a complex issue, particularly for gene expression applications, in which samples contain tens of thousands genes. Shorter oligo probes should theoretically provide higher hybridization discrimination but they tend to have poor hybridization properties leading to lower sensitivity, not to mention the difficulty of finding short unique sequences in large genomes.[7,8] As oligo probes become longer, the hybridization discrimination decreases, although detection sensitivity increases and it is easier to find unique sequences in large genomes. It has been found that when the probe length reaches 35, it needs to have at least 3 mismatches to reliably discriminate different target DNA sequences by hybridization. This fundamental problem of limited specificity has lead to different results from chips of different venders and technology platforms.[9]

[0011] Today's DNA microarrays are not suitable for quantitative measurement. This will likely become one of the roadblocks to hinder the technology from being used as a clinical diagnostic tool, although technological efforts have been made to address this problem.[10] Studies have shown a significant compression of differential ratios (ratios of hybridization intensities from different samples) in microarray data as compared real-time PCR data. Real-time PCR has been established as the most commonly used and accepted standard for validating DNA microarrays in gene expression use.[11] According to the published data, while about 70% of array results of highly differentiated genes were qualitatively consistent with real-time PCR, consistent validation was not achieved for genes showing less than a four-fold change on the array. For many of the genes examined, significant quantitative differences were found between array- and real-time-PCR-based data.[12] For these reasons, array users often choose for further study only those genes with the highest differential expression ratios. This strategy can easily overlook genes of significant interest. Obviously, it is highly desirable to develop a more robust and quantitative array platform in order to reach a level of confidence for which relatively small differences in gene expression between samples are real and that genes showing such differences are worth further investigation.[12]

[0012] The third limitation of today's DNA microarray is detection sensitivity. The single-pair hybridization assay used in the DNA microarray does not involve any amplification and requires a fairly large amount of sample. For example, in gene expression applications with most of the commercial array products, 2 to 5 microgram of total RNA sample is needed for each assay. However, some of the clinical biopsy tissue samples yield less than 1 microgram of total RNA sample. For pathogen detection, microarrays are considered not sensitive enough without the aid of PCR.[13] Amplification of either DNA or RNA samples during sample preparation has been used to boost the amount of samples before they are applied to array chips.[14] This method, however, causes concerns for altering ratios of the genes involved.

[0013] The challenges of specificity, accuracy, and sensitivity mentioned above can be solved using real-time PCR. Higuchi et al. first demonstrated fluorescence monitoring kinetic PCR amplification process in real-time.[15] The method has been developed into a powerful tool, often referred as a golden standard, for quantitative measurement of nucleic acids with various applications, including gene expression, pathogen detection, and SNP (Single Nucleotide Polymorphism) detection. Due to its reduced detection time and simplification of quantification, the method is believed to potentially have the greatest impact on the general public in environmental monitoring and nucleic acid diagnostics.[16]

[0014] A real-time PCR system detects PCR products as they accumulate during a PCR reaction process. There are several variations of detection systems. The most well-known and popular system is Taqman system (C. A. Heid, J. Stevens, K. J. Livak, P. M. Williams, (1996) Real time quantitative PCR. Genome Res. 6, 986).[17] A pair of PCR primers and one fluorescence resonance energy transfer (FRET) probe are used in the detection of each target sequence. The FRET probe is a short oligonucleotide complementary to one of the strands of the target sequence. Each FRET probe contains a reporter dye and a quencher dye. Taq polymerase is used. If the target sequence is present, the probe anneals downstream from the forward primer site and is cleaved by the 5' nuclease activity of Taq DNA polymerase as this primer is extended. The cleavage of the probe separates the reporter dye from quencher dye, increasing the reporter dye signal and allowing primer extension to continue to the end of the template strand. Additional reporter dye molecules are cleaved from their respective probes with each cycle, causing an increase in fluorescence intensity proportional to the amount of amplicon produced.

[0015] Real-time PCR assay is intrinsically highly specific. For one target sequence to be detected, it has to contain all three sequence segments complementary to a detection probe, a forward primer, and a reverse primer, respectively. Any errors produced by one event will likely be filtered out by the other two events. For example, if in one event a forward primer happened to prime to a wrong sample sequence and produced a wrong amplicon, this wrong amplicon will likely either not be recognized by the detection probe or not be further amplified by the reverse primer. In comparison, today's DNA microarrays rely on the hybridization of only one pair of nucleotides and do not have any build-in error-checking mechanism. Even with the multiple-probe approach, such as the one used by Affymetrix,[18] the assay specificity is not increased in any way and the improvement is only on the reduction of the statistical variance of the data. The benefit of this approach is derived by averaging the results of hybridization of multiple individual probes, which hybridize directly with sample sequences and have no relationship with the hybridization events of any other probes that are designed to target at the same sample sequence or same gene.

[0016] Real-time PCR assay is highly sensitive and is quantitative. PCR is an exponential amplification process. In principle, PCR can pick up and amplify a single copy of a target sequence. As a daily practice for RNA detection, real-time PCR requires nanograms of RNA samples as compared to micrograms required by today's DNA microarrays. Moreover, the ability of real-time PCR to quantitatively measure the copy numbers of target sequences in samples is non-existent in today's DNA microarray technology.

[0017] Most of existing instruments perform PCR reactions in either 96- or 384-well titer plates. Samples are manually or robotically pipetted into individual wells. Applied Biosystems recently started the sale of a Micro Fluidic Card in a 384-well format.[19] The new card offers the advantages of reduced consumption of samples and reagents and the elimination of labor-intensive pipetting steps. The new card has the same area size as that of conventional 96- and 384-well titer plates. However, its fluidic design and the operational principle fundamentally limit it from being able to achieve the degree of miniaturization and the level of area density that have demonstrated in DNA microarrays.[20]

[0018] There have been an increasing number of reports of the development of micro-fabricated PCR devices, including continuous flow and microwell devices made from silicon or

plastic materials.[21] A low-energy consumption and fast thermal cycling silicon-chip-based real-time PCR detection system for field use was also demonstrated.[22] There are also reports of performing DNA microarray assays using PCR as a sample preparation process involving microfabricated array chips.[23] However, there has been no published report describing microfluidic real-time PCR microarrays.

[0019] An objective of this invention is to provide microfluidic devices for performing multiplex chemical and biochemical reactions. Another objective of this invention is to provide methods of implanting a plurality of chemical and/or biochemical molecules into the microfluidic devices. Yet another objective of this invention is to provide methods of multiplex biochemical assays using the microfluidic devices. A further objective of this invention is to provide systems for performing parallel chemical and biochemical assay analysis, including real-time PCR and other assays.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0021] FIG. 1A is an exploded perspective view of a chamber array device.

[0022] FIG. 1B is an exploded perspective view of a chamber array device of FIG. 1A that is filled with the first fluid inside chambers as well as transport channels.

[0023] FIG. 1C is an exploded perspective view of a chamber array device that is filled the first fluid inside chambers and the second fluid inside transport channels.

[0024] FIG. 1D is a cross-section view of the chamber array device shown in FIG. 1A.

[0025] FIG. 2A is an exploded perspective view of a chamber array device containing bypass channels that embodies the present invention.

[0026] FIG. 2B schematically illustrates the flow path of the second fluid in the chamber array device of FIG. 2A.

[0027] FIG. 3 is an exploded perspective view of a chamber array device containing serpentine-shaped bypass channels that embodies the present invention.

[0028] FIG. 4A is a schematic diagram of a fluidic device containing tapered channels.

[0029] FIG. 4B is a resistor network model of the fluidic network shown in FIG. 4A.

[0030] FIG. 5A is an exploded perspective view of a chamber array device containing side bypass channels that embodies the present invention.

[0031] FIG. 5B schematically illustrates the flow path of the second fluid in the chamber array device of FIG. 5A.

[0032] FIG. 6A is an exploded perspective view of a capillary array device containing a bypass channel that embodies the present invention.

[0033] FIG. 6B schematically illustrates a cross-section view of the capillary array device of FIG. 6A and the flow path of the second fluid.

[0034] FIG. 7 is a schematic diagram of immobilized oligonucleotides containing multiple segments.

[0035] FIG. 8 schematic illustrates the orthogonal synthesis of two primers and one probe using asymmetric doubler phosphoramidite.

[0036] FIG. 9 is a schematic illustration of the structure of a chip designed for performing parallel synthesis on a bead substrate. For illustration purposes, beads are displayed in only one reaction chamber.

[0037] FIG. 10 is a schematic diagram of a real-time PCR detection system.

DETAILED DESCRIPTION OF THE INVENTION

[0038] Definition of Terms

[0039] The term "photogenerated-reagent precursor" (PRP) refers to a chemical compound that produces one or more reactive chemical reagents when it is irradiated or illuminated with photons of certain wavelengths. The wavelengths may be in any appropriate regions of infrared, visible, ultraviolet, or x-ray.

[0040] The term "photogenerated-acid precursor" (PGAP) refers to a chemical compound that produces acids when it is irradiated or illuminated with photons of certain wavelengths. The wavelengths may be in any appropriate regions of infrared, visible, ultraviolet, or x-ray.

[0041] The term "photogenerated-acid" (PGA) refers to an acid that is produced from PGAP under irradiations or illuminations with photons of certain wavelengths. The wavelengths may be in any appropriate regions of infrared, visible, ultraviolet, or x-ray.

[0042] The term "photogenerated reagent" (PGR) refers to a chemical compound that is produced from the irradiation or illumination of a photogenerated-reagent precursor. In most of the cases, PGR is a reactive reagent in the concerned chemical or biochemical reactions. However, the term may be used to refer to any chemical compounds that are derived from the irradiation of the photogenerated reagent precursor and may or may not be reactive in certain chemical/biochemical reactions.

[0043] The term "probe molecule" refers to a ligand molecule that is employed to bind to other chemical entities and form a larger chemical complex so that the existence of said chemical entities could be detected. Preferably, within a suitable window of chemical and physical conditions, such as pH, salt concentration, and temperature, the probe molecule selectively bind to other chemical entities of specific chemical sequences, specific conformations, and any other specific chemical or physical properties.

[0044] The term "fluid" refers to a liquid or a gas material.

[0045] The term "chamber" refers to a three-dimensional hollow structure that is surrounded by walls of one or more materials. The shape of a chamber may take any forms, include but not limited to cylinder, cube, tube, disk, sphere, hemisphere, or any other regular or irregular three-dimensional forms. A chamber may contain one or more openings.

[0046] The term "aqueous solution" refers to a water solution. The aqueous solution may contain various solutes including but not limited to organic or inorganic salts, organic or inorganic acids, organic or inorganic bases, enzymes, proteins, nucleic acids, surfactants, and other organic or inorganic molecules.

[0047] The term "oil" refers to a liquid that is immiscible or substantially immiscible with water. The oil may be selected from various materials including but not limited to perfluoro compounds, hydrocarbon compounds, mineral oil, and liquid wax..

[0048] The term "fluidic structure" refers to a structure that is constructed or used for handling fluids. A fluidic structure may contain one or more basic components, including but not limited to channels, pipes, slits, chambers, conduits, and holes of various sizes. A fluidic structure may be made of one or more materials selected from various solid as well as flexible materials, including but not limited to glass, plastic, silicon, and elastomer.

[0049] **Approach**

[0050] The present invention provides a novel method and fluidic structures to form a plurality of isolated chambers for the performance of multiplex chemical and biochemical reactions. FIG. 1A is an exploded perspective view of a chamber array device that embodies one aspect of the present invention. The device is made of a fluidic template **110**, on which fluidic structures are fabricated, and a cover plate **140**, which is bonded to the fluidic template **110**. The fluidic structures include chambers **120**, entrant conduits **121**, exit conduits **122**, and transport channels **130**. The sizes, materials, and the relations of the various parts of the disclosed device will become clear as the individual components and the operations of the device are described.

[0051] FIG. 1B and FIG. 1C illustrate the operation process of the disclosed device. In the first step, the first fluid **150** is sent into the device to fill the chambers **120** and transport channels **130**, as shown in FIG. 1B. In the next step, the second fluid **160**, which is immiscible or substantially immiscible with the first fluid, is sent into the device to selectively replace the first fluid **150** in the transport channels **130** while leaving the first fluid **150** in the chambers **120**, as shown in FIG. 1C. As result, the first fluid **150** is confined or isolated inside chambers **120**. The principle and the embodiment fluidic structures to facilitate the selective replacement will be described and become clear in the following paragraphs of this disclosure.

[0052] In a preferred embodiment of the present invention, the first fluid **150** and the second fluid **160** do not or substantially do not chemically interact with each other and immiscible or substantially immiscible with each other. In a one aspect of the present invention, the first fluid **150** is an aqueous solution and the second fluid **160** is oil. The aqueous solution may contain various solutes including but not limited to organic or inorganic salts, organic or inorganic acids,

organic or inorganic bases, enzymes, proteins, nucleic acids, surfactants, and other organic or inorganic molecules. The oil may be selected from various materials including but not limited to perfluoro compounds, hydrocarbon compounds, mineral oil, and liquid wax. In another aspect of the present invention, the first fluid **150** is an aqueous solution and the second fluid **160** is gas. In yet another aspect of the present invention, the first fluid **150** is oil and the second fluid **160** is an aqueous solution. In yet another aspect of the present invention, the first fluid **150** is oil and the second fluid **160** is gas. In yet another aspect of the present invention, the first fluid **150** is gas and the second fluid **160** is an aqueous solution. In yet another aspect of the present invention, the first fluid **150** is gas and the second fluid **160** is oil. Obviously, many more combinations of immiscible fluids can be selected to achieve the isolation of the first fluid **150** inside chambers **120**. For example, an aqueous solution and mercury can be selected as the first fluid **150** and the second fluid **160**, respectively.

[0053] In a preferred embodiment of the present invention, the interior surfaces of chambers **120** and transport channels **130**, shown in FIG. 1A, are coated with films of different affinities. For example, when it is desirable to confine an aqueous solution inside chambers **120**, it is preferred to coat the interior surfaces of the chamber **120**, including upper surface **127**, lower surface **126**, and side surface **125** of FIG. 1D, with a hydrophilic film while coat the interior surfaces of transport channels **130**, including upper surface **137**, lower surface **136**, and side surface **135** of FIG. 1D, with a hydrophobic film. On the other hand, when it is desirable to confine an oil solution inside chambers **120**, it is preferred to coat the interior surfaces of the chamber **120** with a hydrophobic film while coat the interior surfaces of transport channels **130** with a hydrophilic film.

[0054] Construction and Operation of the Disclosed Fluidic Device

[0055] FIG. 2A and FIG. 2B schematically illustrate the structure and operation of an exemplary fluidic device embodiment of the present invention. These drawings reveal the fluid template **210** portion of the device and omit a cover plate for the purpose of visual clarity. Referring to FIG. 2A, when the first fluid is injected into the device, it flows along an inlet distribution channel **271** as an inlet stream **251**, splits into branch streams **252** through inlet transport channels **230**, further splits into chamber streams **253** through entrance conduit **221**, chambers **220**, and exit conduit **222**, merges into branch stream **254** in outlet transport channels

232, further merges into outlet stream **255** in outlet distribution channel **272**, and flows out the device. A port of branch stream **252** passes through a bypass channel **231** to merge into outlet stream **255** without passing through any chamber **220**. A portion of the first fluid **251** in the distribution channel **271** passes through bypass channel **233** to flow through the outlet transport channel **232** and makes up a part of the branch stream **254**. In one aspect of the present invention, the fluidic structures of the fluidic device of FIG. 2A are symmetric so that inlet and outlet of the device can be switched without effecting fluid flow characteristics except the reversal of flow directions. In the most preferred embodiment of the present invention, the cross-section area of the bypass channels **231** and **233** is significantly larger than that of the inlet conduit **221** of the chambers **220**.

[0056] FIG. 2B illustrates the flow of the second fluid through the fluidic device of the present invention. For explanation purpose, we assume that the first fluid **250** is an aqueous solution and has already filled the fluidic device before the second fluid is injected into the fluidic device. We further assume that the interior surfaces of chambers **220** are hydrophilic. Under these assumptions, in a preferred embodiment of the present invention the second fluid is either a gas or oil and the interior surfaces of fluid channels are hydrophobic. When the second fluid is injected into the fluidic device, under an appropriate flow rate, it inters the inlet distribution channel **271** as an inlet stream **261**. A portion of the inlet stream **261** flows into an inlet transport channel **230** to become a branch stream **262**, which passes through an bypass channel **231** and merges into an outlet stream **265** in an outlet channel **272**. Another portion of the inlet stream **261** passes through a bypass channel **233** and flows along an outlet transport channels **233** as a branch stream **264** and then merges into the outlet stream **265** in the outlet channel **272**. During this process the second fluid pushes the first fluid out of the fluidic device everywhere except chambers **220**. As a result, the first fluid is isolated inside the chambers **220**.

[0057] The operational principle of the fluidic device of this invention is based on pressure barriers at the junctions of cross section change. Assume a channel having a hydrophilic internal surface, a cross-sectional area of A, a wetted perimeter of L, and is filled with water. According to Shaw in "Introduction to Colloid and Surface Chemistry" Butterworths, London, 1983, the minimum pressure required to push air into this channel is estimated by $P = \frac{\gamma \times L}{A}$, where γ = 72.8 mN/m is the surface tension of water at water/air interface. As shown in FIG. 2B and FIG.

2A, the wetted perimeters through the inlet conduit **221** of a chamber **220** and through a bypass channel **231** of the inlet transport channel **230** are $L_c = 2(W_c + H_c)$ and $L_b = 2(W_b + H_b)$, respectively. W_c, W_b and H_c, H_b, are the width and the height of inlet conduit **221** and bypass channel 231, respectively. The corresponding cross-sectional areas are $A_c = W_c \times H_c$ and $A_b = W_b \times H_b$, respectively. For explanation purpose, we assume that $W_c = 28$ μm, $H_c = 14$ μm, $W_b = 48.5$ μm, $H_b = 150$ μm. We then derive that the minimum pressures for air to push through the inlet conduit **221** and the bypass channel **231** are $P_c = 2.26$ psi and $P_b = 0.57$ psi, respectively. Therefore, as long as we send in an air with a pressure between 0.57 psi and 2.26 psi we will push water out of the inlet transport channel **230** through bypass channel **231** but not chamber **220** through inlet conduit **221**. We call this pressure range as operational pressure window. Obviously, it is desirable to have a wide operational pressure window. We also assume that bypass channel **233** has the same cross section dimensions as that of bypass channel **231** so that in the same pressure range air would pass through the bypass channel **233** and push water out of the outlet transport channel **232**. As result, water is isolated by air on both inlet and outlet sides of the chamber **220**. The above analysis is based on a simplified calculation to serve the purpose of explanation. More elaborated calculations are available such as the one by Man et al. in "Microfabricated capillarity-driven stop valve and sample injector", at 1998 MEMS Conference, Heidelberg, Germany, Jan. 25-29 1998.

[0058] Based on the principle that are described above, those skilled in the art of fluidics are able to perform calculations to estimate the operation conditions and to vary fluidic structures to achieve the isolation of fluid inside chambers when different fluids are used. Calculations relating to fluidic flow through fluidic structures that are coated with films of different affinities are also well known to those skilled in the art (Man et al. in "Microfabricated plastic capillary systems with photodefinable hydrophilic and hydrophobic regions", at the 1999 Transducers Conference, Sendai, Japan, June. 7-10 1999).

[0059] FIG. 3 shows another preferred fluidic device embodiment of the present invention. Serpentine-shaped bypass channels **331** are utilized so that the total length of the bypass channels **331** can be adjusted to achieve a suitable ratio between the amounts of fluid **353** flown through chambers **320** and fluid **356** flown through the bypass channels **331** while minimizing the size of the fluidic device. For many assay applications of the fluidic device of this invention, some of which are described in later sections of this disclosure, it is desirable to maximize the flow

through the chambers **320** or to minimize the flow through the bypass channels **331**. On the other hand, as described in the above paragraphs, it is desirable for the bypass channels **331** to have large cross-section areas so as to obtain a wide operational pressure window. Therefore, it is often desirable to increase the total length of the bypass channels **331** in order to reduce the flow through the bypass channels **331** while using a reasonably large cross-section area for the bypass channels **331**. The calculation of fluid flow in a fluidic network, such as the fluidic device of this invention, is a well-known art to those skilled in the art of fluid dynamics.

[0060] FIG. 4A shows a schematic diagram of another preferred fluidic device embodiment of the present invention. In this embodiment, fluidic channels are shaped in such a way that predetermined flow rate distributions across the fluidic channels and chambers **420** are obtained. For example, it is often desirable to have a uniform flow across all chambers **420**. In case of the fluidic device shown in FIG. 4A, this means that the volume flow rates of chamber streams **461**, **462**, **463**, **464**, **465**, and **466** are identical. This invention achieves this by using tapered fluid channels as shown in FIG. 4A. The shapes of the tapered inlet distribution channel **471** and outlet distribution channel **472** are designed to distribute a fluid into and out of transport channels **451**, **455**, **454**, and **458** according to predetermined ratios. In one exemplary design, the volume flow rate of stream **455** equals to that of stream **454**, the volume flow rate of stream **451** equals that of **458**, and the volume flow rate of stream of **451** is half of that of steam **454**. The shapes of the transport channels **430**, **433**, **434**, and **437** are designed to produce uniform volume flow rate across all chambers **420**.

[0061] For a given fluid flow distribution, the shapes of fluid channels can be designed based on fluidic dynamic calculation and/or mathematical modeling which are well-known to those skilled in the art of fluidics. One simple and effective modeling approach is resistor network calculations. This approach is valid under steady state laminar flow conditions. FIG. 4B shows a resistor network model of the fluidic device of FIG. 4A. Each resistor represents one segment of the fluidic structure. For example, resistors $R_{I1\text{-}1}$, $R_{I1\text{-}2}$ to $R_{I1\text{-}8}$ of FIG. 4B make up the inlet transport channel **430** of FIG. 4A. Resistor $R_{I1\text{-}9}$ of FIG. 4B represents the bypass channel **431** of FIG. 4A. $R_{C1\text{-}1}$, $R_{C1\text{-}2}$, and $R_{Ci\text{-}j}$ (where i = 1 to 3 and j = 1 to 8) of FIG. 4B represent chambers **420** of FIG. 4A. The resistance is defined as the ratio of pressure drop and volume flow rate. The calculation of pressure drop through various fluidic structures, such as rectangle channel, slab, and pipe, is familiar to those skilled in the art of fluid dynamics and can be found in

literature such as the one by White "Fluid Mechanics", 3rd ed. John Wiley and Sons, (1994) and the references therein. For a given flow condition, for example an equal volume flow rate through resistors R_{Ci-j} for i = 1 to 3 and j = 1 to 8 R, a set of simultaneous linear equations are established. More than one solution may be derived by solving the equations when the number of unknowns is more than the number of equations, meaning that more than one set of fluidic structural parameters can be used to achieve the same basic fluid flow condition, such as uniform flow through all chambers **420** of FIG. 4A. With additional conditions, such as fixing the values of R_{I3-9}, forcing $R_{I3-9} = R_{O2-1}$, $R_{I3-8} = R_{O2-2}$, ..., $R_{I1-9} = 2R_{I3-9}$, $R_{I1-8} = 2R_{I3-8}$, ..., a unique solution can be found, from which the shapes of the inlet and outlet transport channels **430**, **434**, **433**, and **437** of FIG. 4A are derived. Obviously, following the above teaching, those skilled in the art can design fluidic structures to achieve predetermined flow distributions other than uniform flow across chambers **420** of FIG. 4A. Commercial computational fluidic dynamic software packages, such as FLUENT from Fluent Inc., New Hampshire, USA and CFD-ACE from CFD Research Corporation, Alabama, USA, are available and can be used for simulating fluid flow so as to help the design of fluidic structures of the present invention.

[0062] FIG. 5A and FIG. 5B illustrate the structure and operation of yet another preferred fluidic device embodiment of the present invention. Key fluidic structures of this device include chambers **520**, inlet transport channels **530**, outlet transport channels **532**, and bypass channels **522**. This embodiment differentiates from the one shown in FIG. 2A and FIG. 2B in the arrangement of bypass channels. As shown in FIG. 5A, in this fluidic device embodiment each chamber **520** is surrounded by a bypass channel **523** while the fluidic device shown in FIG. 2A the bypass channels **231** and **233** are placed at the end of the inlet and outlet transport channels **230** and **232**. However, the operation principles of the two embodiments are the same. In a preferred embodiment, the across-section area of the bypass channel **523** is substantially larger than that of the inlet conduit **521** of chamber **520**.

[0063] To operate the fluidic device of FIG. 5A, the first fluid is initially sent into the fluidic device through an inlet distribution channel (not shown in FIG. 5A). The first fluid splits into branch streams **552** and flow along inlet transport channels **530**, further splits and flows through bypass channels **523** and through inlet conduits **521**, chambers **520**, and outlet conduits **522**, and merges into branch streams **554** in outlet transport channels **532**, and eventually merges into an outlet distribution channel (not shown in FIG. 5A) and exits the fluidic device. Referring to FIG.

5B, after the fluidic device is filled with the first fluid **550**, the second fluid, which is immiscible with the first fluid **550**, is sent into the fluidic device. The second fluid splits into branch streams **562** and flows along the inlet transport channels **530**; it further splits, flows through bypass channels **523**, and merges into branch streams **564** in the outlet channels **532**. The second fluid would not pass through the inlet conduit **521** under the following illustrative conditions. First, the first fluid **550** is an aqueous solution. Second, the interior surface of the chambers **520** is hydrophilic. Third, the second fluid is either a gas or oil. Forth, the across-section area of the bypass channel **523** is substantially larger than that of the inlet conduit **521**. In addition, the flow rate of the second fluid needs to be sufficiently low so that the pressure drop between the junctions of inlet conduit **521** and the outlet conduit **523** at the bypass channel **523** is lower than a surface-tension induced pressure barrier at the entrance cross-section of the inlet conduit **521**. As result, the first fluid **550** is isolated inside the chambers **520**. In this operation embodiment we place no restriction on the flow directions of either the first or the second fluid.

[0064] FIG. 6A the structure of yet another preferred fluidic device embodiment of the present invention. The device is composed of a fluidic template **610**, a side enclosure **644**, a top enclosure **640**, and a bottom enclosure **642**. The fluidic template **610** contains a plurality of capillary chambers **620** and a bypass channel **631**. There are inlet and outlet holes **641** and **643** on the top and the bottom enclosures **640** and **642**, respectively for delivering a liquid into and out of the device. The operational principle of this fluidic device embodiment is the same as what is described in the above paragraphs. In one illustrative embodiment the first fluid is an aqueous solution, the second fluid is a gas, the internal surface of the capillary chambers **620** is hydrophilic, and the cross-section area of the bypass channel **631** is much larger than that of capillary chambers **620**. As shown in FIG. 6B, after the device is filled with the first fluid **650**, the second fluid **661** is sent in through an inlet hole **641**. Inside the device, the second fluid replaces the first fluid in top gap **630**, the bypass channel **631**, and the bottom gap **642** while leaving the first fluid **650** isolated inside the capillary chambers **620**.

[0065] Fabrication

[0066] In a preferred embodiment, the fluidic template **110** of FIG. 1A is made of silicon material and is formed using fabrication processes, such as photolithography, etching, and coating, which are well-know to those skilled in the art of microfabrication (Madou, M.,

Fundamentals of Microfabrication, CRC Press, New York, (1997)). In one aspect of the present invention, the surface of the fluidic template 110 is preferably coated with silicon dioxide, which can be made by either oxidation or evaporation during a fabrication process.

[0067] In another preferred embodiment, the fluidic template **110** is made of plastic materials, including but not limited to polyethylene, polypropylene, polystyrene, polycarbonate, polydimethylsiloxane, polyamide, polymethylmethacrylate, polyoxymethylene, epoxy, polyvinylidine fluoride, and polytetrafluoroethylene. A plastic fluidic template **110** can be made using a fabrication process selected from or combined of molding, embossing, casting, laser abolition, and mechanical machining methods, which are well-know to those skilled in the art of plastic processing as described by Becker et al. in "Polymer microfabrication methods for microfluidic analytical applications". Electrophoresis 21, 12-26 (2000) and the references therein. The use of plastic materials often has the advantage of low cost and ease of production.

[0068] Varieties of other materials, such as ceramic, glass, metal and composites of two or more materials, and corresponding fabrication processes can also be used to make the fluidic template **110**.

[0069] The capillary fluidic template **610** shown in FIG. 6A can be made from silicon material by a high aspect ratio etching process using commercial equipment such as ASE etching system supplied by Surface Technology Systems, Newport, UK. The capillary fluidic template **610** can also be made from glass materials using ultrasonic drilling, laser drilling and any other appropriate fabrication processes that are well-known to those skilled in the art of microfabrication.

[0070] In one aspect of this invention, the cover plate **140** of FIG. 1A is a flat and transparent plate. The use of a transparent cover plate **140** is required when a chamber array device shown in FIG. 1A is used as a multiplexing photochemical reactor or as an assay device involving photo-detections. When a fluidic template **110** is made of Si, the cover plate **140** is preferably made of glass, which is anodically bonded to the Si fluidic template **110**. Exemplary glass materials include but not limited to Corning 7740 (from Corning Incorporated, Corning, NY 14831) and Borofloat® (from Schott Corporation, Yonkers, NY 10701). Plastic materials can also be used to make the cover plates **140**. Plastic cover plates **140** can be attached to fluidic templates **110** using an appropriate bonding processes selected from but not limited to gluing,

heating, laser welding, and lamination which are well-known by those skilled in the art of plastic processing.

[0071] In another aspect of this invention, the cover plate **140** contains structural features that are not shown in FIG. 1A. For example, chambers **120** shown in FIG. 1A may be made on the cover plate **140**. In this case, the cover plate **140** becomes the second fluidic template to make up a complete fluidic structure after combining the cover plate 140 with the fluidic template **110**.

[0072] The selective coating of the interior surfaces of chambers **120** and channels **130** of FIG. 1A with films of different affinities can be achieved with various methods that are familiar to those skilled in the art of surface chemistry and microfabrication. In one illustrative silicon-based fabrication process, the silicon fluidic template **110** is first coated with silicon dioxide using an oxidation process. Then, the chambers **120**, inlet conduits **121**, and outlet conduits **122** are coated with a photoresist film by using a photolithography process while leaving the interior surface of channels **130** exposed. The exposed channel **130** surfaces are coated with a hydrophobic film by dipping the silicon template into an alcohol solution of a fluorinated silane compound. When the photoresist film is removed with acetone the exposed silicon dioxide interior surfaces of chambers **120** and conduits **121** and **122** are hydrophilic. Other methods, including photochemical methods, can also be used to selectively tailor the surface affinities.

[0073] While there is no fundamental limitation on the size of the fluidic structures of the present invention, the preferred distance between adjacent chambers **120** is in the range of 1 to 5,000 μm. More preferably, the distance is in the range of 10 to 2,000 μm. Yet more preferably, the distance is in the range of 10 to 500 μm. Even more preferably, the distance is in the range of 10 to 200 μm.

[0074] **Applications**

[0075] A preferred application embodiment of the present invention is multiplexing bio assay, including but not limited to real-time PCR, hybridization, immunoassay, ELISA, and peptide or protein binding assay. The present invention provides novel devices and methods for achieving a significantly increased degree of multiplexing for these assays as compared to the currently available technologies.

[0076] Real-time PCR is a well-known bio assay method to those skilled in the art of molecular biology (C. A. Heid, J. Stevens, K. J. Livak, P. M. Williams, (1996) Real time quantitative PCR. Genome Res. 6, 986.). For real-time PCR assay use, the cover plate **140** of FIG. 1A is preferably transparent. Each chamber **120** is first deposited with a pair of sequence specific primers. For some assays, such Tagman real-time PCR, a probe is also needed in the chamber **120**. Methods primer and probe deposition are described in the flowing paragraphs. Then, a sample solution containing sample DNA or RNA sequences, polymerase enzymes, NTPs, and other necessary reagents, is injected into the fluidic device. The next step is the isolation of the sample solution into individual chambers by injecting an isolation fluid into the device. The isolation fluid is either a hydrophobic liquid or an inert gas. The isolation prevents the diffusion or exchange of any molecules among individual chambers during the following thermal cycling PCR reaction. Then the real-time PCR reaction can be performed in a way that is essentially the same manner as a regular real-time PCR process. The thermal cycling for the PCR reaction can be performed using Peltier thermoelectric device with thermal couple or thermistor sensors for temperature measurement and feedback control. Mercury or Xenon lamps equipped with proper filters, lasers, or LEDs can be used as the light source for the excitation of fluorescence dyes. Photomultiplier and CCD can be used to detect the emissions from the fluorescence dyes. Laser scanning instruments or their variations that have been used for collecting fluorescence images from DNA and other microarrays can be used for collecting fluorescence images from the fluidic devices of the present invention. The instrumentation and the performance of real-time PCR process are well-know to those skilled in the art of analytical instrumentation and molecular biology.

[0077] Various methods that are well-know to those skilled in the art of microarrays can be used to deposit primers and probes into chambers. Two exemplary methods are spotting and in situ synthesis. For spotting, primers and probes may be either covalently bonded to a substrate surface or non-covalently deposited to the substrate surface. For the covalently bonded primers and probes, it is preferred that these surface bonded molecules contain cleavable sites so that they can be cleaved from the substrate surface before or during a PCR reaction. For the non-covalently deposited primers and probes, it is preferred that measures are taken to prevent the molecules from being washed away from reaction chambers when a PCR mix solution is being filled into the chambers. One exemplary measure is to mix the primers and probes with an

agarose gel of an ultra-low gelling temperature so that the primer or probe oligos will not be washed away by the PCR mix solution and will become available in solution phase for the PCR reaction when the device is heated up.

[0078] A preferred embodiment of the present invention is a novel real-time PCR assay method utilizing the fluidic device of the present invention. This new assay method combines hybridization and PCR to achieve high sensitivity and high specificity. Probe molecules containing multiple segments of nucleotides are deposited or synthesized on a substrate **710** surface as shown in FIG. 7. In a fluidic device of the present invention the substrate **710** surface of FIG. 7 is the interior surface of chambers **110** of FIG. 1A. In a preferred embodiment, the probe molecule consists of three nucleotide segments, including a forward primer **772**, a reverse primer **774**, and a binding probe **776**. At the bottom of each probe molecule is a linker **770** segment which bridges between the probe molecule and the substrate **710**. The above segments are connected by cleavable sites **771**, **773**, and **775**. The sequence design of forward and reverse primers **772** and **774** can may follow the same principles as that of regular real-time PCR such as the ones summarized by Bustin "Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays", Journal of Molecular Endocrinology (2000) 25, 169–193. In a preferred embodiment of the present invention, the binding probe should have a Tm of about 10°C or higher than that of primers. The liner segment **770** can be an alkyl, polyethylene glycol, or various other chemical linker moieties that are familiar to those skilled in the art of solid phase synthesis and microarrays. The cleavable sites **771**, **773**, and **775** may include U nucleotide, reverse U nucleotide, disthio group and other chemical moieties that can be cleaved by enzymes, chemicals, light, and any other means that do not cause any adverse effect to PCR reactions.

[0079] In an illustrative hybridization-PCR assay embodiment of the present invention primers **772** and **774** and binding probe **776** have an orientation of 3' to 5' from the lower to the upper sides of the probe molecule. Cleavable sites **771** and **773** are reverse U and **775** is U. With this probe molecule structure, PCR active 3' hydroxyl groups will be produced in primers **772** and **774** and PCR inactive 3' phosphate group will be produced in binding probe **776** after the probe molecule is subjected to RNase A. In an assay process, a solution containing single-strand DNA sample sequences is first circulated through the fluidic device at a proper temperature so that those sample sequences complimentary to respective binding probes **776**

would be hybridized and retained in the corresponding chambers while non-specific sample sequences would not be retained. A brief wash with an appropriate buffer solution will then be applied to the fluidic device at a reduced temperature to wash the non-specific sample sequences out of the device while keeping the hybridized sample sequences in the chambers. This washing step further improves the specificity of the PCR assay. A PCR mix based on SYBR Green I double-stranded DNA binding dye assay is then be injected into the device. In a preferred embodiment RNase A is used to cut the cleavable sites **771**, **773**, and **775** and release primers **772**, and **774** and binding probe **776** into solution. To avoid premature enzymatic cleavage, chip temperature will be kept low (e.g. at 4°C) when the RNase A containing PCR mix is injected into the device. An isolation fluid is then injected into the chip to isolate all the chambers and real-time PCR reaction is carried out. The hybridization process enriches specific sample sequences into corresponding small chambers and therefore significantly increases the assay sensitivity. At the same time non-specific sequences is washed out of chambers and therefore the chance for mis-priming during PCR is significantly reduced and the assay specificity is significantly improved. It should be noted that in this assay 3' ends of binding probes **776** is blocked so that they will not become PCR primers during PCR reaction.

[0080] ***Primer/Probe Attachment and Release***: In situ parallel synthesis involving photogenerated acid will be used to attach primers and probes to silicon-based microfluidic array chips. One special requirement for real-time PCR use is to have all three oligos attached to the same reaction cell. We achieve this by synthesizing all three oligo segments in one combined sequence with adjacent segments separated by a cleavable reverse U (rU) nucleotide as shown in FIG. 7. As we described earlier in Preliminary Studies section, rU can be cleaved by RNase A, which can be mixed into PCR solution without effecting PCR reactions. The total length of the combined sequence is between 60 to 100 nucleotides, which is well within the capability of our synthesis chemistry. Our chemistry has also been demonstrated to be able to synthesize double-dye probes, which are required by Taqman assay. FIG. 8 shows an alternative orthogonal synthesis approach for making three oligo segments. We use an asymmetric doubler phosphoramidite (supplied by Glen Research, Virginia), which contains one acid-labile DMT protected branch and one base-labile Fmoc protected branch, to synthesize two primers on one branch and one probe on the other branch.

[0081] Our in situ synthesis on the surface of the chip is capable of producing more than sufficient amounts of oligos for real-time PCR use. From our previous studies we estimate oligo surface density to be between 0.05 to 0.2 pmole/mm^2. At this surface density, after cleavage we can obtain an oligo concentration between 4 to 16 μM in a reaction cell of 25 μm deep (note: each cell contains an upper and a lower internal surface). In standard real-time PCR protocols, optimal primer concentration is between 0.1 to 1.0 μM and probe concentration is about 0.05 μM.[Error! Bookmark not defined.] Obviously, we would have more materials than what is needed in the reaction cells. We reduce the oligo solution concentration either by making deeper reaction cells or by intentionally reducing oligo surface density. In our previous studies (not published) we have develop a method of controlling oligo surface density by using a coupling solution containing a mixture of a regular phosphoramidite and a "terminating" phosphoramidite at a predetermined ratio. The method can be used to separately control the densities of primers and probes (in either regular or orthogonal synthesis of FIG. 7 and FIG. 8) to be optimized for real-time PCR reactions.

[0082] The process of releasing oligos from surface can be selected from several alternatives. We have developed several different 2'-protected uridine phosphoramidites for improving enzymatic cleavage efficiency. For example, we have successfully synthesized 2' FpMp (1-(2-Fluorophenyl)-4-methoxy-1, 2, 5, 6-tetrahydropyridine) protected reverse U phosphoramidite, which has a theoretical cleavage efficiency of 100% using a RNase enzyme.

[0083] ***Real-time PCR assay***: Although using our microfluidic array PCR chip we will be able to perform almost all the existing real-time PCR assays, the new device that contains probes and primers on the reaction cell surfaces (see FIG. 7 and FIG. 8) will allow us to perform a new and extremely sensitive and highly specific **hybridization – PCR assay**. The new assay is suitable for detecting single strand DNA or RNA sequences. Each assay would require three oligos, including one probe, one forward primer and one reverse primer. The design of primers and probes for this assay would be similar to that for Taqman assay.[19] The probe should have a Tm about 10°C higher than that of primers. A solution containing sample sequences will first be circulated through the chip at a proper temperature so that those sequences complimentary to respective probes are hybridized and retained in the corresponding reaction cells while non-specific sequences are not retained. A brief wash will then be applied to the chip at a reduced temperature to wash the non-specific sequences out of the chip while keeping the hybridized

sequences in the reaction cells. This washing step further improves the specificity of the PCR assay. A PCR mix based on SYBR Green I double-stranded DNA binding dye assay will then be injected into the chip. The only new content in the PCR mix is RNase A, which is used to cut the primer/probe combined sequences at rU sites forming component primers and probes (FIG. 7 and FIG. 8). To avoid premature enzymatic cleavage, chip temperature will be kept low (e.g. at 4°C) when the RNase A containing PCR mix is injected into the chip. An isolation fluid will then be injected into the chip to isolate all the reaction cells and real-time PCR reaction will be carried out. The hybridization process will enrich specific sample sequences into corresponding small reaction cells and therefore significantly increase the assay sensitivity. At the same time non-specific sequences will be washed out of reaction cells and therefore the chance for mis-priming during PCR is significantly reduced and the assay specificity is significantly improved. It should be noted that in this assay 3' ends of probes need to be blocked so that the probes will not become primers during PCR reaction. This can be easily accomplished by replacing rU at the 3' end of probes shown in FIG. 7 and FIG. 8 with U during the synthesis process. We will carry out studies to compare the proposed new assay with the established real-time PCR assays. For carrying out standard real-time PCR assays, the hybridization step is not necessary. Sample sequences can be incorporated into PCR mix and together be injected into a chip.

[0084] Those skilled in the art of molecular biology should be able to map out an operational window of the real-time PCR device and associated assays. Among the variable parameters are the effect of probe density, the order of primer/probe in the combined sequences (FIG. 7 and FIG. 8), and the geometry of the reaction cell. Other parameters include PCR additives, such as BSA (bovine serum albumin) and PEG (polyethylene glycol).

[0085] Another preferred application embodiment of the present invention is parallel assays involving chemluminescence and/or bioluminescence, such as ELISA and hybridization. In these applications, a solution containing enzyme (such as horseradish peroxidase) attached target samples (antibody, protein, DNA, or RNA) are first circulated through a microfluidic array device of this invention that contains probes (peptides, DNA, or RNA). A substrate solution containing luminol, hydrogen peroxide, and an enhancer is then injected into the microfluidic array device. Nitrogen is then passed through the chambers of the microfluidic array device so as to isolate reaction chambers. Chemical luminescence signal is then collected using a cooled

CCD camera or a photomultiplier-based measurement instrument. The reaction-chamber isolation eliminates diffusion of substrate during chemluminescence reaction.

[0086] Another preferred application embodiment of the present invention is the multiplexing chemical reaction and/or synthesis. The present invention provides improvements to earlier disclosed technologies, such as the one disclosed by Zhou in "Fluidic Methods and Devices for Parallel Chemical Reactions" PCT WO 0202227, by introducing a new and simple isolation mechanism. In one aspect of the present invention, photogenerated reagents in solution phase and projected light patterns are used to facilitate chemical reactions in a plurality of selected chambers 120 of FIG. 1A simultaneously. The method and the apparatus relating to the use of the photogenerated reagents are described by Gao in "Method and Apparatus for Chemical and Biochemical Reactions Using Photo-Generated Reagents" US 6,426,184. One important aspect of the method is to confine active photogenerated reagents inside individual chambers 120 of FIG. 1A so as to prevent the active reagents from going from a light-exposed chamber into neighboring chambers due to diffusion effect.

[0087]

[0088] The use of bypass channels in the present invention permits the isolation of reaction solution inside reaction chambers. In one illustrative embodiment of the present invention, a solution containing photogenerated reagent precursor is first injected into a fluidic device, such the one shown in FIG. 2A, of this invention. An inert gas, such as helium, is then sent into the device to push the solution out of distribution, transport, and bypass channels 271, 272, 230, 231, 232, and 233 so as to isolate the solution inside the chambers 220. A selected number of chambers 220 are then exposed to light so as to generate activate reagents inside the exposed chambers 220. After a period of time that is sufficient for the completion of the intended chemical reactions inside the chambers 220, a wash solution is sent into the device to flush the active reagents out the device. This new isolation mechanism is particularly useful for those applications that require extended reaction time after the photogenerated active reagents are generated by light. The fluidic device of the present invention can be used to synthesize microarrays of various chemical and biochemical molecules, including but not limited to DNA, RNA, peptide, carbonhydride, and the combination of the above molecules. In a preferred

embodiment of the present invention, the probe molecules of FIG. 7 are synthesized using the chemical method involving light generated reagents.

[0089] ***Instrumentation***: FIG. 10 shows a real-time PCR system for performing fluidic circulation, thermal cycling, and optical detection. The system consists of a fluid station for injecting samples/PCR mix and isolation fluid into a microfluidic array chip, a Peltier thermoelectric heating/cooling unit for performing thermal cycling on the microfluidic array chip, a filtered illumination system for exciting fluorescence dyes inside the microfluidic array chip, a cooled CCD camera for detecting fluorescence emission from the microfluidic array chip, and a computer controller. For the filtered illuminator, a mercury lamp can be used. In another preferred embodiment and a blue LED based optical system can be used.[33] For SYBR Green I and FAM excitation and detection we use a bandpass filter at 475 nm as exciter and bandpass filter at 535 nm as emitter.[34] A high sensitivity 14-bit cooled CCD camera can be used for fluorescence detection. A scanning detection system can also be for the detection.

[0090] Variations and Modifications

[0091] In another aspect of this invention, the cover plate **140** is a flat and opaque or translucent plate. The optical transparency of the cover plate **140** is not necessary when a chamber array device shown in FIG. 1A is to be used as a multiplexing reactor for non-photochemical reactions and a non-photo-detection based assay device. An exemplary non-photochemical reaction is electrochemical reaction, which has been described by Montgomery in U.S. Patent No. 6,444,111. By adding electrodes in a chamber array device, one skilled in the art of electrochemistry may perform multiplexing synthesis reactions. An exemplary non-photo-detection based assay is the electron transfer based nucleic acid detection, which is described by Meade et al. in U.S. Patent No. 6,013,459 and the references therein. By adding electrodes in a chamber array device, one skilled in the art of molecular electronic detection may perform multiplexing nucleic acid and other molecular detection.

[0092] Another preferred variation of the present invention is the use of a microwell plate to perform hybridization-PCR assay. In a preferred embodiment a microwell plate contains a plurality of microwells of 1 to 500 microns in diameter and 1 to 500 microns deep. The plate can be made of glass, silicon, plastic, and any other appropriate materials. The fabrication of such a plate is well-know to those skilled in the art of microfabrication. In a preferred embodiment, the

microwell plate is assembled with an enclosure to form a fluidic device which contains inlet and outlet to allow fluids to be injected and/or circularted. An exemplary make and use of a glass-based microwell plates is described by Leproust et al. in "Digital light-directed synthesis. A microarray platform that permits rapid reaction optimization on a combinatorial basis", J. Comb. Chem. 2, 349-354 (2000). For real-time PCR application, the bottom of the wells is covalently deposited with probe molecules containing primers and binding probes. In a prefered embodiment, the interior surface of the microwells is hydrophilic and the outside surface of the microwells hydrophobic. In an illustrative assay process, a solution containing single-strand DNA sample sequences is first circulated through the fluidic device at a proper temperature so that those sample sequences complimentary to respective binding probes would be hybridized and retained in the corresponding microwell while non-specific sample sequences would not be retained. A brief wash with a suitable buffer solution will then be applied to the fluidic device at a reduced temperature to wash the non-specific sample sequences out of the device while keeping the hybridized sample sequences in the microwells. A PCR mix based on SYBR Green I double-stranded DNA binding dye assay is then be injected into the device. As described in the above paragraphs, the PCR mix contains RNase A or other appropriate cleavage reagents. To avoid premature cleavage, chip temperature will be kept low (e.g. at 4°C) when the PCR mix is injected into the device. An isolation fluid, such as oil, is then injected into the chip to isolate all the microwells and real-time PCR reaction is carried out thereafter.

[0093] Another alternative form of microwell plates is to facilitate a different isolation mechanism. Each microwell has an extruded lip. The microwells can be sealed or isolated by pressing an elastomer sheet or a laminate film having an adhesive coating against the microwells. The extruded lip helps the seal. The elastomer and the laminate film can be selected from various materials that are compatible with the temperatures used in PCR processes, chemically inert, and of low fluorescence.

[0094] Another aspect of the present invention is the implementation of beads materials into the fluidic device to significantly increase the synthesis capacity of the device in parallel synthesis applications. In a preferred embodiment the beads are made of high-loading substrate materials including but not limited to partially crosslinked and functionalized polystyrene beads, crosslinked polystyrene-PEG copolymer beads, CPG, and various other commonly used and specialized resin material used in solid phase synthesis. In a preferred embodiment, all beads are

substantially spherical and of narrow size distribution. A fluidic device similar to that shown in FIG. 2A, except the structure of the reaction chambers, is used. In one aspect of the present invention the outlet side of each reaction chamber contains a fence to stop beads from passing through and allow liquid to flow through. The bypass channels should be wide enough to allow beads to pass through so as to avoid plugging of the transport channels by the beads. Before loading the beads into the reaction chambers, the beads are suspended in a liquid having substantially the same density as that of the beads (excluding the void inside the beads). Then, the bead suspension liquid is circulated through the fluidic device till all the reaction chambers are filled with the beads. The process of using the bead-loaded fluidic device for chemical synthesis is similar to that of a regular device as what is described in the above paragraphs.

[0095] FIG. 8 schematically illustrates an exemplary structure of a bead-containing chip. For illustration purpose, only a 1D array is shown. A 2D array, which is the format of a real chip, can be constructed by repeating the 1D structure in the y direction. During an operation, the fluid enters the chip through a main inlet channel, splits and flows into branch inlet channels (there are multiple inlet branch channels in a 2D array device), further splits and flows through reaction cells, merges into branch outlet channels, and further merges into a main outlet channel and flows out of the chip. A portion of the incoming fluid reaches the main outlet channel through inlet or outlet bypass channels without passing through any reaction cells or the branch inlet channels. The function of the bypass channels will be described later. The main considerations in the design of this chip include fluid flow distribution, synthesis capacity, bead-loading mechanism, chemical and photochemical reaction efficiency, device fabrication, and production cost.

[0096] For fluid flow distribution, we use tapered fluid channels to produce a uniform flow across all reaction chambers along the channels. The shape of the channels is derived by using a mathematical model based on resistor networks as we described in this disclosure. The fluidic flow inside the device is laminar flow and the flow resistance through the channels and reaction chambers is calculated using the established formulations in fluidic mechanics.[35] Such a modeling procedure has been successfully used in the design of our commercial microfluidic array chips and validated by both CFD (Computational Fluidic Dynamics) simulation and tracing experiments.[36]

[0097] The synthesis capacity is determined by the quantity and the capacity of beads in each reaction chamber. Considering that the size and the packing density of the beads will affect fluid flow and synthesis protocols, we will design, fabricate, and test chips for accommodating beads of various sizes (e.g. 10, 20, and 35 μm) and select the most suitable size. The selection is mainly based on the quality of the synthesized product as determined by chemical analyses. In a preferred embodiment, a relatively small number of beads are used in each reaction chamber. For example, only 20 to 25 20-μm beads will be needed in each reaction chamber to produce 10 pmol of a 60-mer oligo, assuming a stepwise yield of 99% for the synthesis and 1.0 pmol loading capacity for each bead. Since the 20 μm beads will swell to 27 μm during synthesis reactions, net area of each reaction chamber needs to be 18,000 μm^2 (assuming a single layer of beads in each reaction chamber). To include the areas required for channels and dividing barriers between adjacent reaction cells, we estimate the gross area of each reaction chamber to be twice the bead occupancy area. For a chip to accommodate 10,000 reaction cells, the size of such a chip would be 360 mm^2 or 19 mm × 19 mm, which is the right size for a microfabricated device to be economically manufactured. The total internal volume of such a chip will be less than 20 μL. Therefore, the **reagent consumption for chemical synthesis in such a chip will not be more than that in a regular 1-μmol column** which usually has a total internal volume of 200 μL.

[0098] Since only a limited number of beads are going to be packed into each reaction chamber of ~0.5 nL volume, we do expect statistic variations of packing density and the consequent variations of flow resistance through the packed beads in different reaction chambers. We reduce the impact of this variation to the flow rate distribution by incorporating grooves at the bottom and the top surfaces of each reaction chamber. The bottom grooves are shown in FIG. 8. When properly designed, these grooves will provide a constant path for fluid to flow by the beads and through the reaction chambers. The overall resistance of each reaction chamber is determined by the parallel connected "resistors" of packed beads and micromachined grooves. When the resistance of grooves is sufficiently small, the resistance variation of the packed beads would have insignificant effect to the overall resistance of the reaction cell. The grooves also provide an anti-clogging mechanism. The function of the grooves is much more than just as flow resistance reducer, they provide a critical transportation path for the delivery of

reagents to the beads. The grooves produce micro-reaction conditions for the beads inside the reaction chambers similar to that in a float-bed reactor, which is commonly used for solid-phase reactions. The ultimate criteria for determining the uniformity of the reaction conditions across the chip are the consistency in quality and the quantity of the oligo products from individual reaction chambers.

[0099] Several measures can be taken to ensure beads are retained inside reaction chambers during synthesis. Our preliminary results demonstrate that the fence structure is effective in preventing the beads from flowing through in the forward direction. The remaining issue is to prevent the beads from moving backwards and fall out of reaction chambers. For this, first, a forward flow direction will be maintained throughout the synthesis process so that there is no driving force for the beads to flow backwards. Second, after the beads are loaded into reaction cells, a thorough wash of the chip is performed to dislodge any loose beads at the entrance of the reaction chambers. Third, we use micromachined gate structures at the entrance of the reaction chambers which would allow beads to flow in but make it difficult for beads to flow back out. One such structure, as illustrated in FIG. 8, is a cylindrical rod that narrows the entrance of the reaction chamber. In actual devices, other shaped objects, such as chevrons pointing outwards, could form more effective gate.

[00100] Another important fluidic structure for the microfluidic bead chip is the bypass channels shown in FIG. 8. In a bead loading process, a bead suspension is circulated through a chip and some of the beads are carried into and accumulate inside reaction chambers along the branch inlet channels while the remaining beads are carried through the bypass channels and flushed out the chip. The circulation continues until all reaction chambers are fully filled with beads. The cross-section of the bypass channel is larger than the beads to avoid any clogging or accumulation of the beads in the branch channels. For bead loading, the bypass outlet channel is not necessary. However, it provides a means to adjust fluid flow distribution. For example, during a synthesis process it is desirable to quickly flush PGA out of the branch outlet channels and an adequate amount of fluid coming through the bypass outlet channels would be helpful to increase the flow rate in the branch outlet channels. Although straight bypass channels are depicted in FIG. 8, serpentine shaped bypass channels may be used in actual chip devices so as to increase the length of the bypass channel for reducing the fluid flow through the channel while keeping the required cross-section area for beads to pass through.

[00101] Another aspect of the present invention that fluidic structures may be fabricated on one continues substrate or constructed from individual fluidic components, such as vessels, tubes, and connectors.

[00102] We claim

[00103] 1. A fluidic device containing bypass channels

[00104] 2. A fluidic device containing isolation mechanism for real-time PCR application

[00105] 3. A fluidic device containing isolation mechanism for chemluminescence assay application

[00106] 4. An assay method combining hybridization with PCR

[00107] 5. A fluidic device containing bypass channels for bead loading and reaction applications

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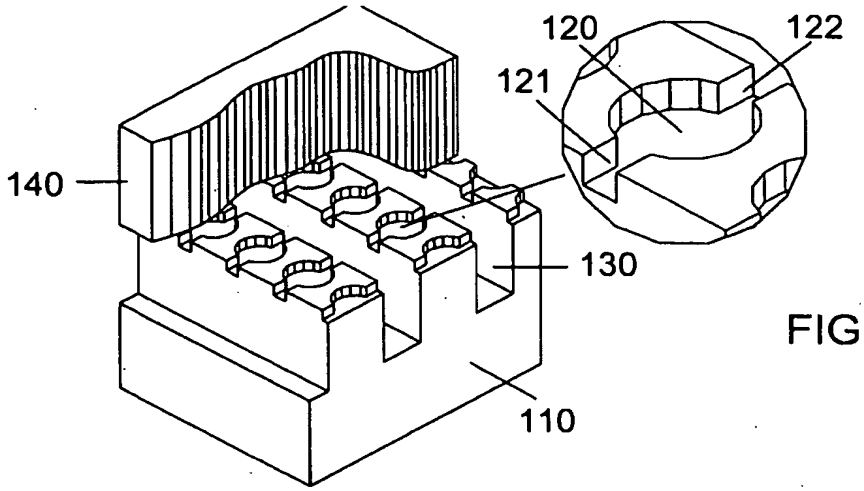


FIG. 1A

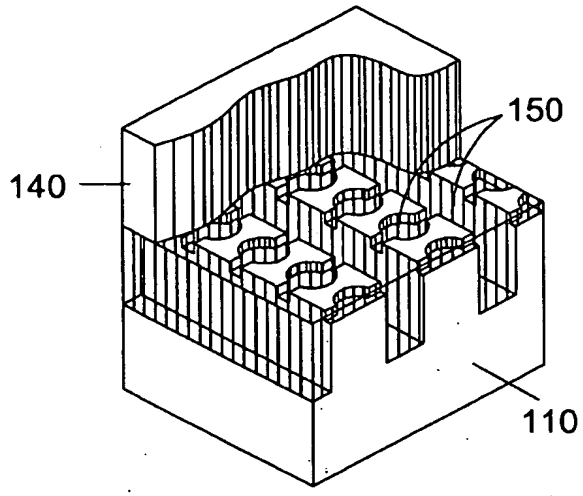


FIG. 1B

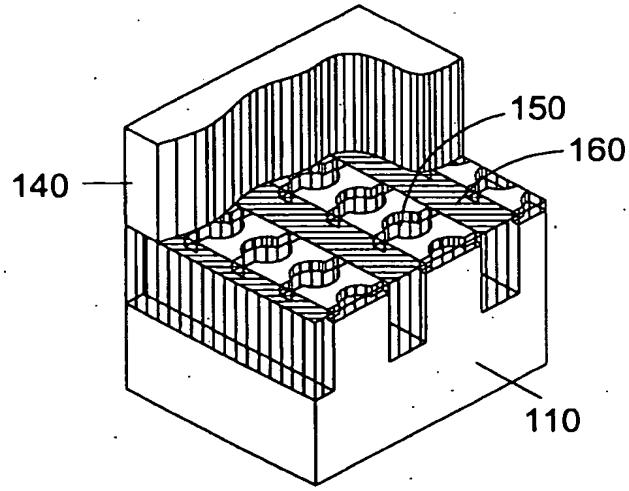
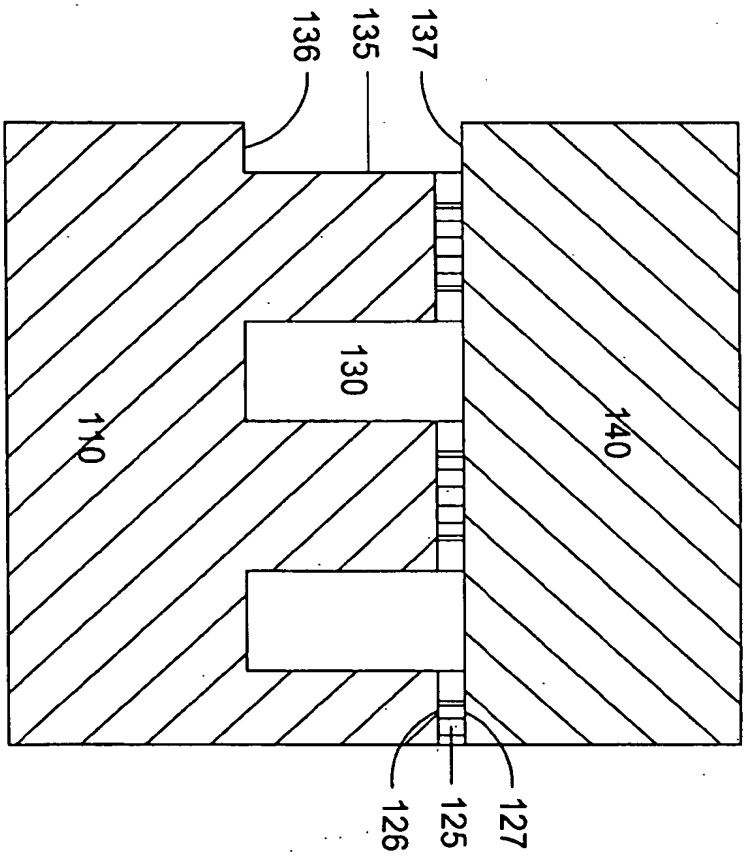


FIG. 1C



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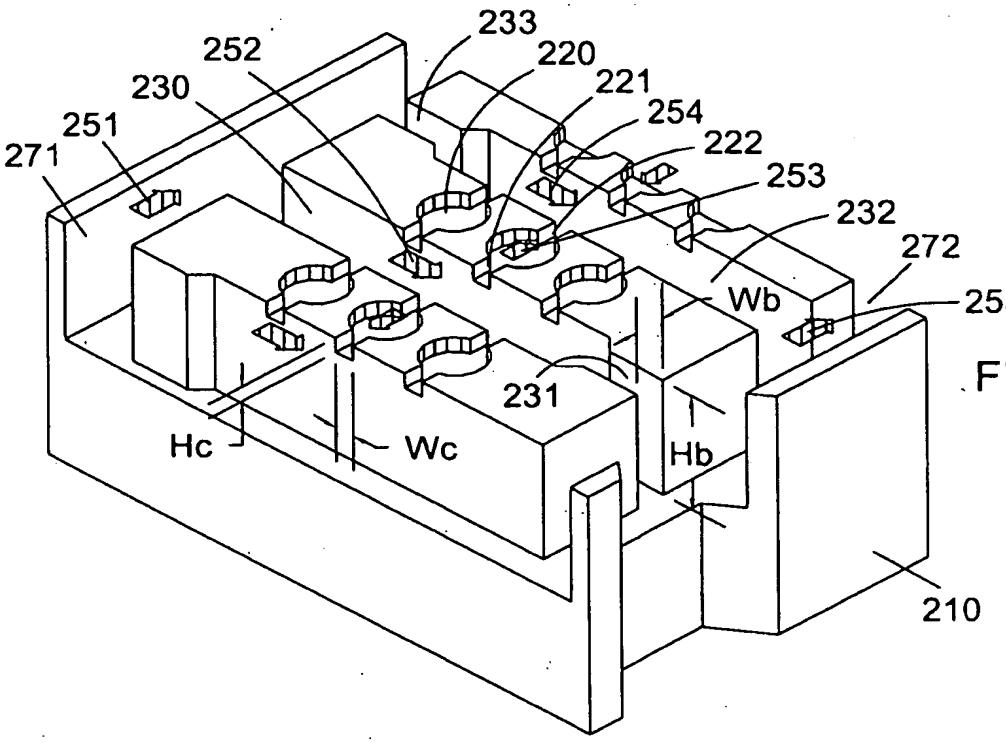
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Wb
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Hc
Wc
Hb
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FIG. 2A

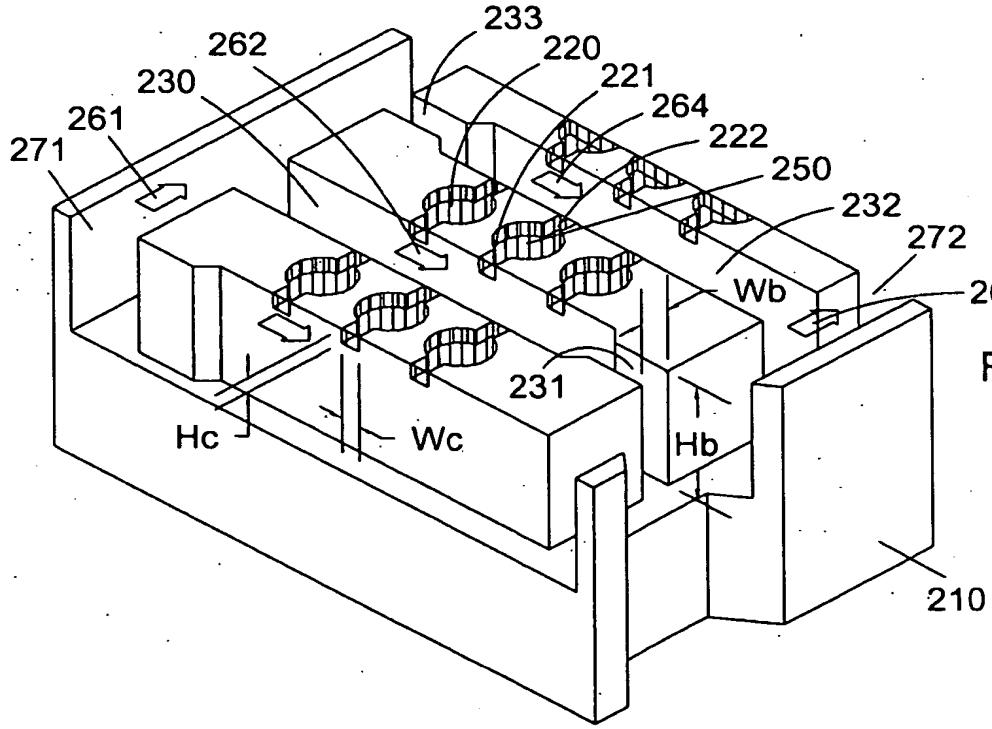
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FIG. 2B

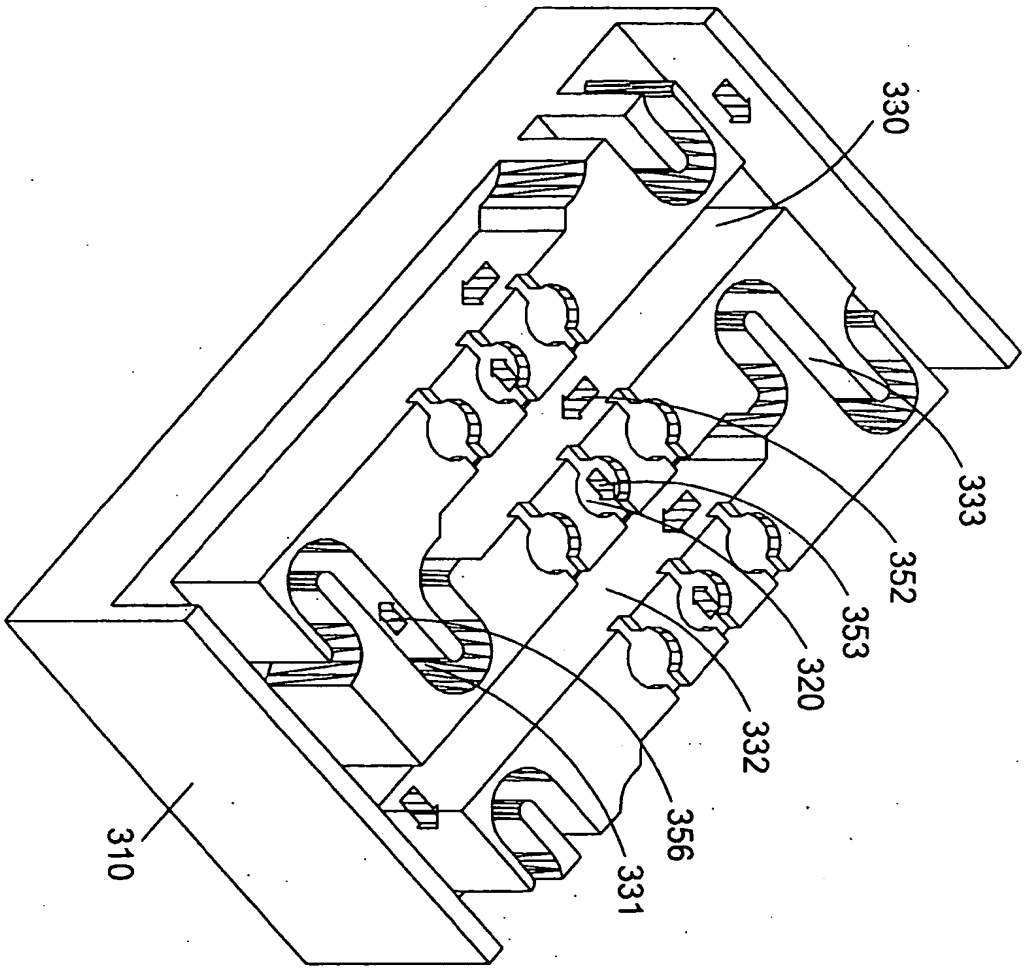

FIG. 3

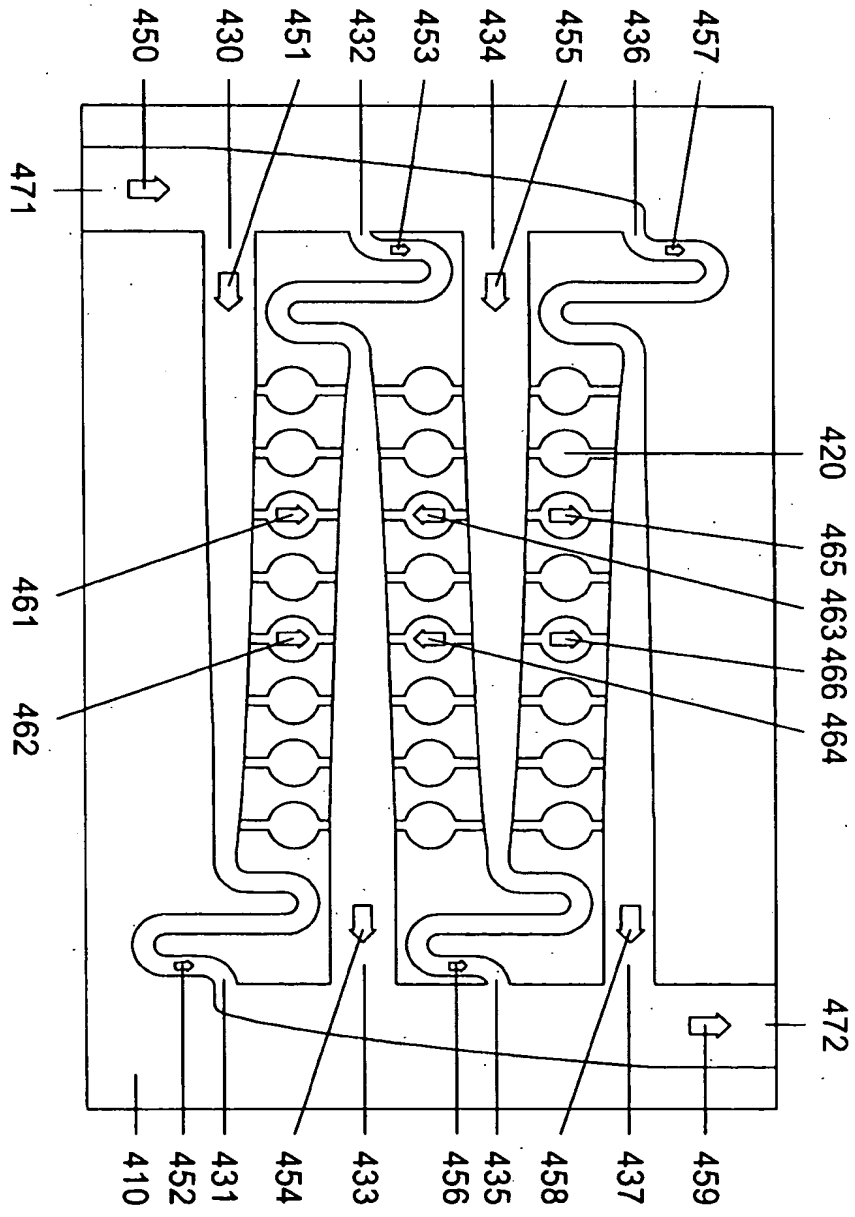
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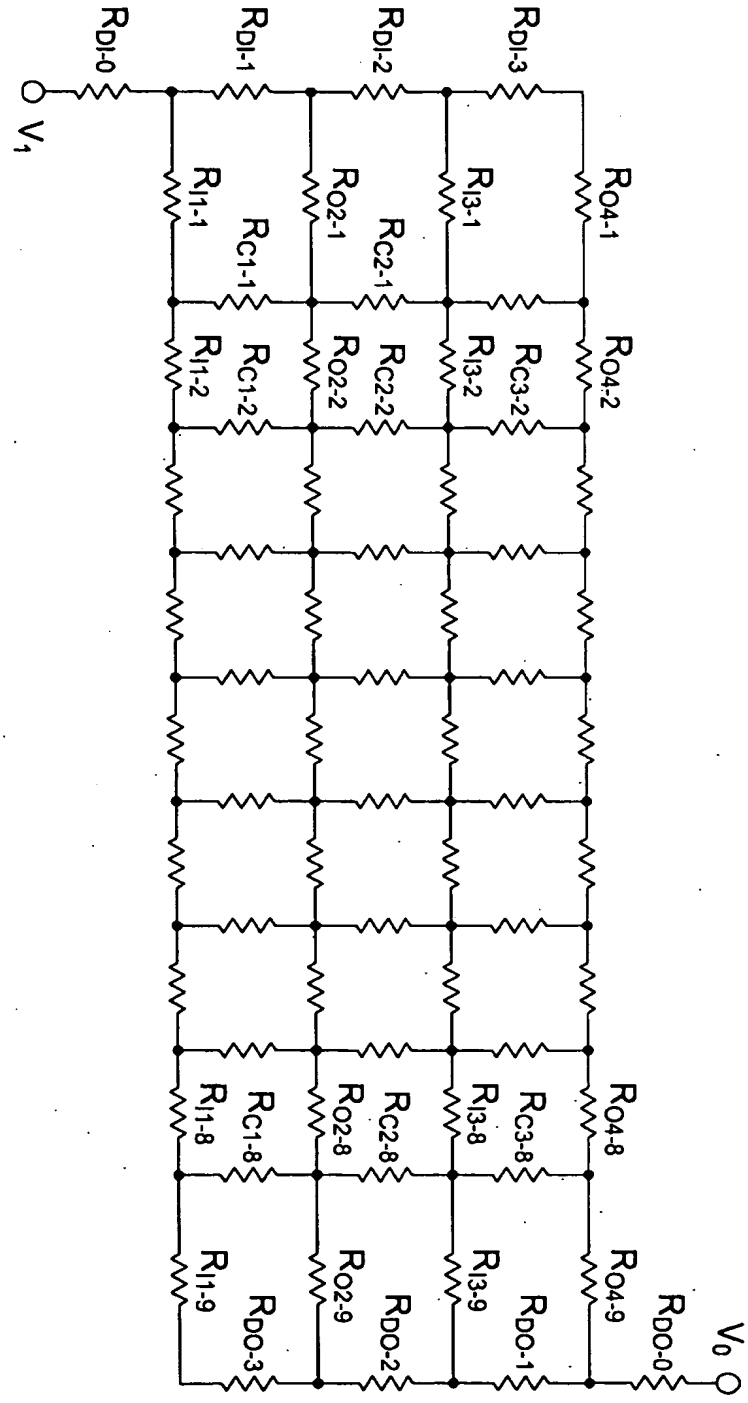
FIG. 4A


R_{DI-0}
R_{DI-1}
R_{DI-2}
R_{DI-3}
V_1
R_{I1-1}
R_{C1-1}
R_{O2-1}
R_{C2-1}
R_{I3-1}
R_{O4-1}
R_{I1-2}
R_{C1-2}
R_{O2-2}
R_{C2-2}
R_{I3-2}
R_{C3-2}
R_{O4-2}
R_{I1-8}
R_{C1-8}
R_{O2-8}
R_{C2-8}
R_{I3-8}
R_{C3-8}
R_{O4-8}
R_{I1-9}
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R_{O4-9}
R_{DO-0}
V_0

FIG. 4B

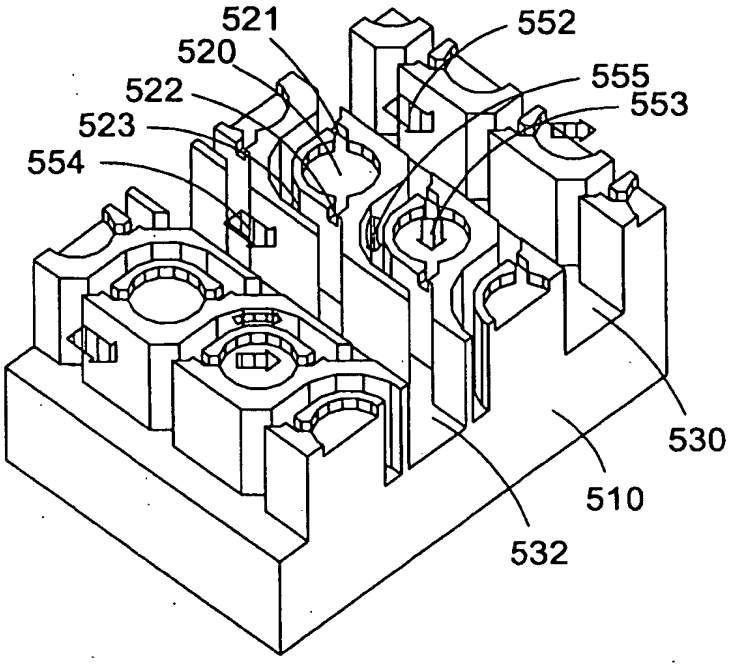
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FIG. 5A

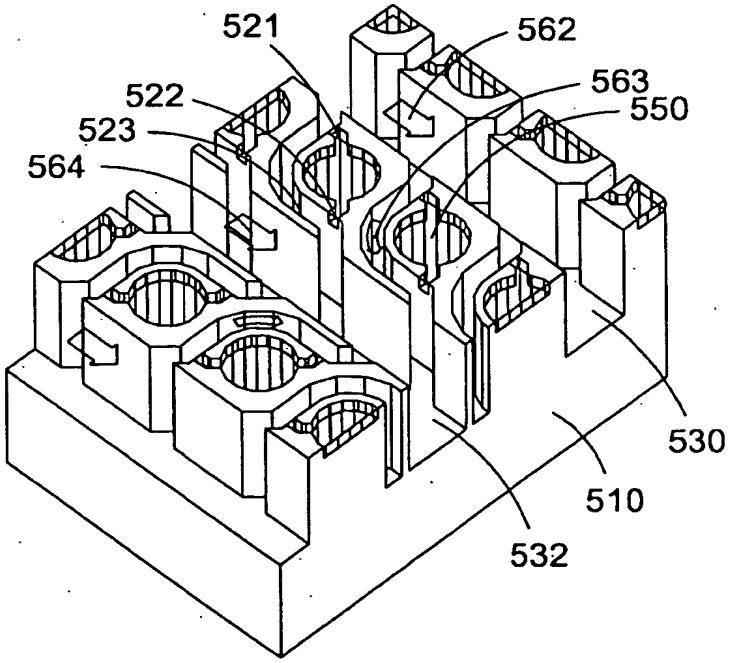
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FIG. 5B

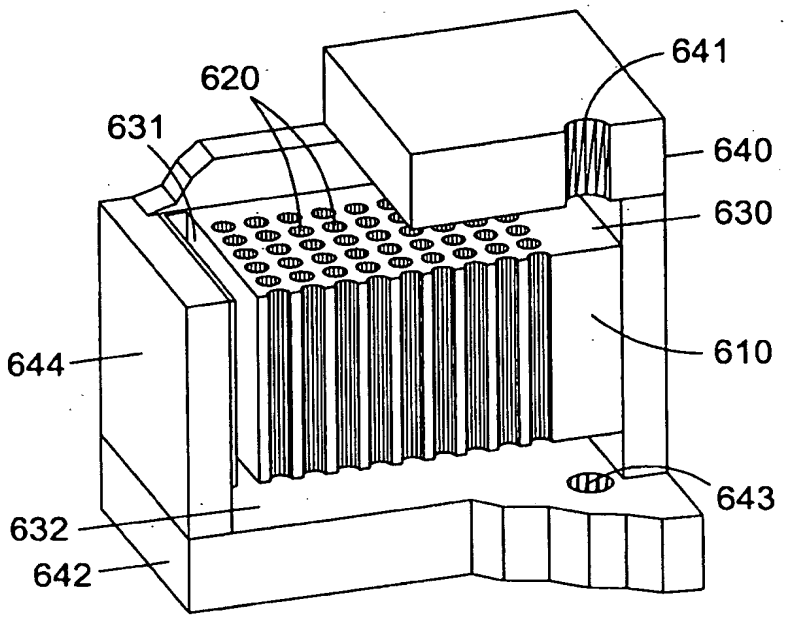
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FIG. 6A

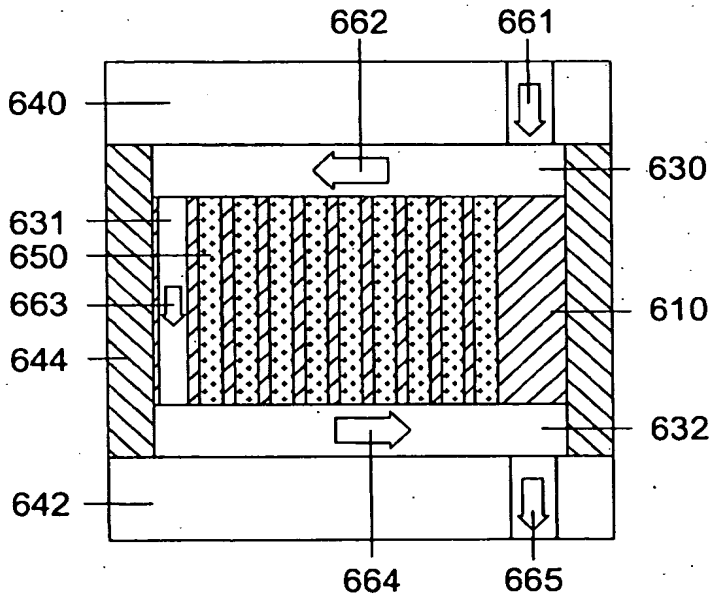
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FIG. 6B

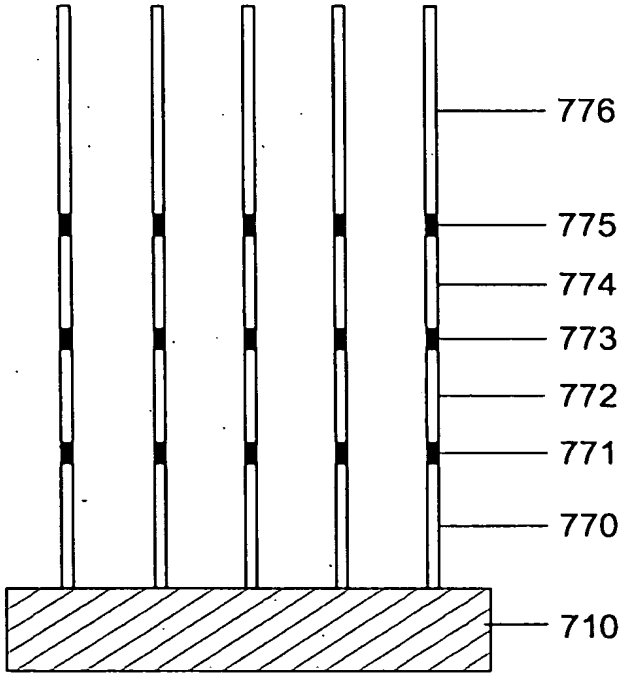

FIG. 7

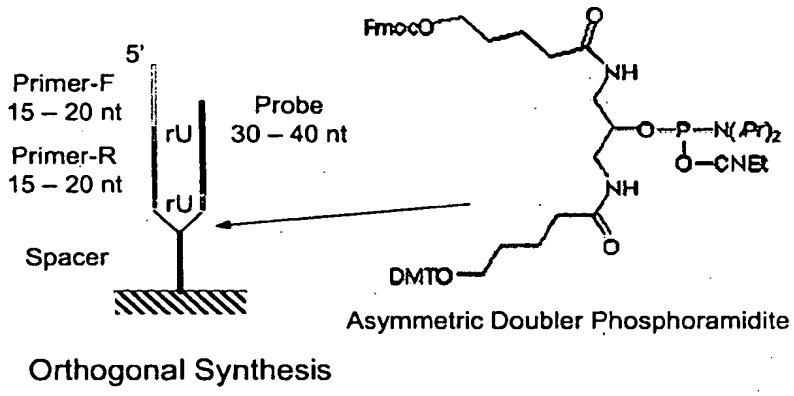
5'
Primer-F
15 – 20 nt
rU
Probe
30 – 40 nt
Primer-R
15 – 20 nt
rU
Spacer
FmocO
NH
O
O—P—N(iPr)2
O—CNEt
NH
O
DMTO
Asymmetric Doubler Phosphoramidite
Orthogonal Synthesis

FIG. 8

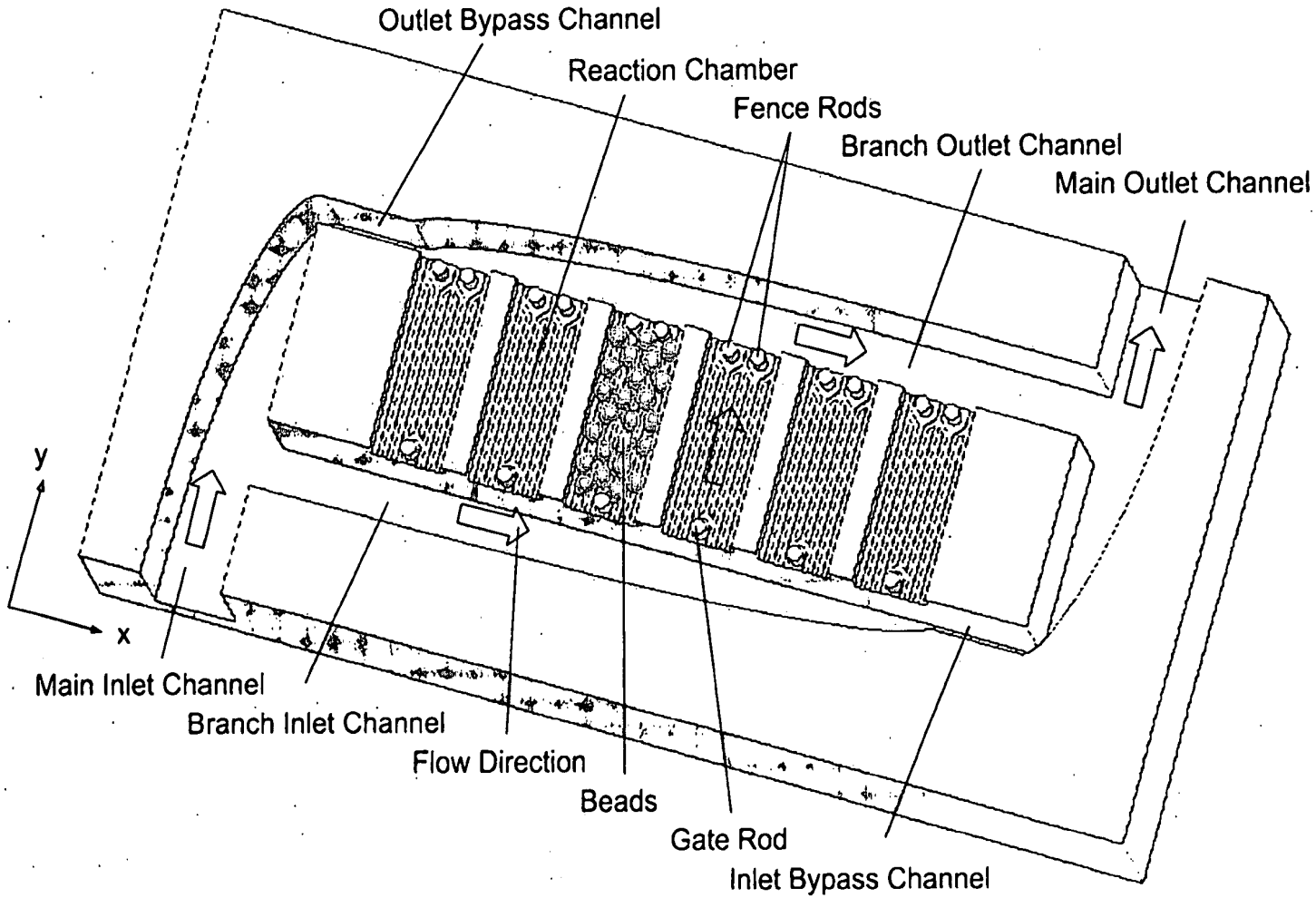
Outlet Bypass Channel
Reaction Chamber
Fence Rods
Branch Outlet Channel
Main Outlet Channel
y
x
Main Inlet Channel
Branch Inlet Channel
Flow Direction
Beads
Gate Rod
Inlet Bypass Channel

FIG. 9

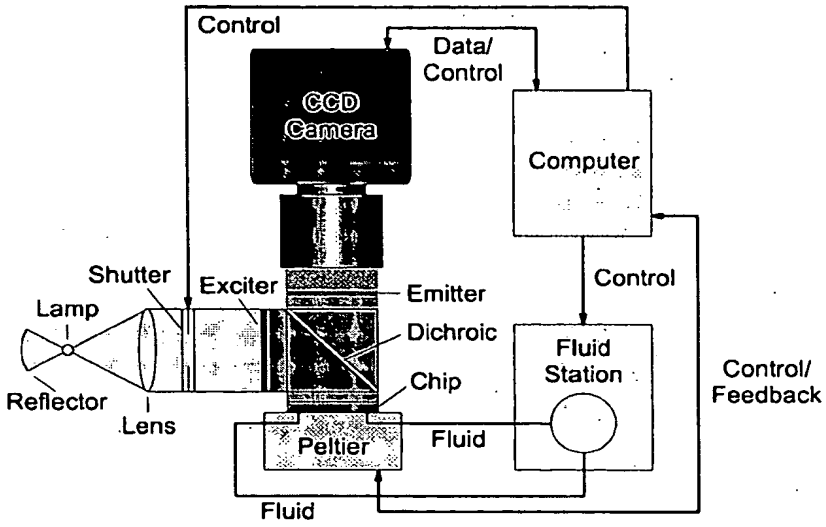
Control
Data/
Control
CCD
Camera
Computer
Shutter
Exciter
Emitter
Control
Lamp
Dichroic
Fluid
Station
Control/
Feedback
Chip
Reflector
Lens
Peltier
Fluid
Fluid

FIG. 10

PATENT COOPERATION TREATY

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PCT

NOTIFICATION CONCERNING
SUBMISSION OR TRANSMITTAL
OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

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2212B Bellefontaine Street
Houston, TX 77030
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)* 28 April 2005 (28.04.2005)	
Applicant's or agent's file reference ATA04001-PCT	**IMPORTANT NOTIFICATION**
International application No. PCT/US05/005389	International filing date *(day/month/year)* 18 February 2005 (18.02.2005)
International publication date *(day/month/year)*	Priority date *(day/month/year)* 18 February 2004 (18.02.2004)
Applicant ZHOU, Xiaochuan et al	

1. By means of this Form, which replaces any previously issued notification concerning submission or transmittal of priority documents, the applicant is hereby notified of the date of receipt by the International Bureau of the priority document(s) relating to all earlier application(s) whose priority is claimed. Unless otherwise indicated by the letters "NR", in the right-hand column or by an asterisk appearing next to a date of receipt, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. *(If applicable)* The **letters "NR"** appearing in the right-hand column denote **a priority document which, on the date of mailing of this Form, had not yet been received by the International Bureau** under Rule 17.1(a) or (b). Where, under Rule 17.1(a), the priority document must be submitted by the applicant to the receiving Office or the International Bureau, but the applicant fails to submit the priority document within the applicable time limit under that Rule, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

3. *(If applicable)* **An asterisk (*)** appearing next to a date of receipt, in the right-hand column, denotes **a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b)** (the priority document was received after the time limit prescribed in Rule 17.1(a) or the request to prepare and transmit the priority document was submitted to the receiving Office after the applicable time limit under Rule 17.1(b)). Even though the priority document was not furnished in compliance with Rule 17.1(a) or (b), the International Bureau will nevertheless transmit a copy of the document to the designated Offices, for their consideration. In case such a copy is not accepted by the designated Office as the priority document, Rule 17.1(c) provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
18 February 2004 (18.02.2004)	60/545,435	US	23 March 2005 (23.03.2005)

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